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Prevalence and spectrum of dermatophytes and non-dermatophyte mold isolated from patients with Tinea Capitis, attending Rank Higher Specialized Dermatology Clinic, Addis Ababa, Ethiopia.

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This is to certify that the thesis is prepared by Betelhem Yilma, entitled:

“Prevalence and spectrum of dermatophytes and non-dermatophytes mold isolated from patients with Tinea Capitis, attending Rank Higher Specialized Dermatology Clinic, Addis Ababa, Ethiopia” and submitted in partial fulfillment of the requirements for the Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public health microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
LPCB	Lacto phenol Cotton Blue
KOH	Potassium Hydro-Oxide
QC	Quality Control
Spp.	Species
SDA	Sabouraud dextrose agar
SPSS	Statistical Packages for Social Sciences
WHO	World Health Organization

Operational Definition

Dermatomycosis: -a disease (as ringworm) of the skin caused by infection with a fungus also called epidermomycosis.

Dermatophytes: - a fungus parasitic upon the skin or skin derivatives (as hair or nails). It is fungal organisms that require keratin for growth.

Dermatophytosis: - a disease (as athlete's foot) of the skin or skin derivatives that is caused by a dermatophyte

Mycoss: - infection with or disease caused by a fungus.

Tinea/***ringworm*****:** -any of several contagious diseases of the skin, hair, or nails of humans and domestic animals caused by fungi (as of the genus *Microsporum*, *Trichophyton*, and *Epidermophyton*) and characterized by ring-shaped discolored patches Tinea Capitis on the skin that are covered with vesicles and scales.

Tinea Capitis: - an infection of the scalp caused by fungi of the genera *Trichophyton* and *Microsporum* and characterized by scaly patches Tinea Capitis penetrated by a few dry brittle hairs

Non-dermatophyte molds: fungi with known found in nature as soil saprophytes and plant debris.

Kerion: -a severe pustular inflammation involving the hair follicles

Biosafety level: - laboratory designation determined by risk assessment of the pathogenicity of the agent manipulated and nature of work performed.

Abstract

Background: In the past decades, scalp ringworm or Tinea Capitis has become a major public health problem affecting the developing countries. Tinea Capitis is a fungal infection that predominantly affected scalp and hair shafts. The distribution, epidemiology, frequency, clinical manifestations, and target hosts varies location to location. According to various studies, Tinea Capitis has been affecting mostly school age children. This harms the psychological and emotional aspects of those children lives.

Objective: The objective of our study was to determine the extent of Tinea Capitis and spectrum of dermatophyte and non-dermatophyte molds implicated in causing fungal infection.

Material and Methods: A prospective non-randomized study was conducted at Rank Higher Specialized Dermatology Clinic, from February 2019 to July 2019. A total of 364 study participants were collected by convenient sampling techniques. For this study, socio-demographic data was collected. Then fungal pathogen from Scalp Scraped samples was identified by using KOH microscopic examination. Those samples were inoculated on to mycological culture media. All inoculated plates were incubated at appropriate temperature for at least for four weeks. Fungi were identified based on their microscopic, macroscopic and cultural characteristics. The sample data were analyzed using IBM SPSS statistics version 23 software.

Results: In our study, the overall prevalence of Tinea Capitis 301/364(82.7%). Dermatophytes were isolated in136 (45.2%) and the most frequent pathogen was *Trichophyton violaceum* followed by non-dermatophyte molds 121 (40.2%) *Aspergillus fumigatus* was the most common species and mixed infections were observed in 44 (14.6%) cases. Among 301 positive subjects the predominant age group 1-14(84.4%) years followed by25-44 (10%).

Conclusion: High prevalence of Tinea Capitis infection with *Trichophyton violaceum* was the predominant etiological agent in this study. This shows that Tinea Capitis is still a public health problem. There is a need for public awareness of the problem and develop a capacity for personal hygiene.

Keywords: Tinea Capitis, Dermatophytes, non-dermatophytes, prevalence

1. Introduction

1.1Back ground

Dermatophytosis is a fungal infection caused by a group of fungi that have a tendency to colonize and infect the keratinized layer of the skin or skin derivatives that is caused by a group closely related fungi called dermatophytes [1].

Dermatophytosis has been distributed throughout the world, but it is more prevalent to tropical regions. Warm and moist weather conditions help the growth of dermatophytosis [1]. Factors such as living condition, immigration history, definitive host range, intermediate host immunity and access to health care for on time treatment are attributed to the variability of the worldwide distribution of dermatophytes [2].

Dermatophyte is a group of three genera *Trichophyton,Microsporum* and *Epidermophyton.* Two of these genera *(Trichophyton* and *Microsporum)*are the most causative agents of Tinea Capitis. Dermatophytes is thought to spread mainly from person-to-person (anthropophilic), from infected animals (zoophilic), and from soil (geophilic). It is also spread indirectly from fomites [3]. The recent increase in systemic diseases compromises the immune system like diabetes. This caused in suppression of host immune defense mechanisms by chronic medical condition exposed to humans more at risk not only to infectious fungi but also to all fungi that were once regard as foreign matter [4].

According to different countries studies dermatophytes and non-dermatophyte fungi involved as a cause of dermatophytosis; however, how often diseases occur in different group of people, incidence, clinical symptom and target hosts varies country to country. This difference is due to geographical locations, health care, environmental factors, culture and socioeconomic conditions [5].

Tinea Capitis is a fungal infection specifically involving affecting the skin of the scalp and hair. It is the most common dermatophyte infection with prevalent in sub-Saharan African descents, particularly in children. The clinical presentation of Tinea Capitis varies, to some extent, by the etiologic agent. T. tonsurans infection can present in a number of different ways, including

inflammatory and non-inflammatory. Hair loss, scaling, impetigo like plaques and erythema are the common signs of Tinea Capitis. Tinea Capitis also affects adults but it is not common. Limited treatment options and various modes of transmission complicate the clinician's ability to address this disease appropriately. Although dermatophytes are prevalent in our environment, Tinea Capitis one of the widespread, therapeutic options that can be developed to reduce morbidity [6].

Tinea Capitis is a common problem in many African countries and its prevalence varies between 10% and 30%, It is believed that approximately 20 million active infections with an even higher carriage ratio. The widespread of the infection caused by distinct organisms have been described, for example, *Microsporum audouini* in Nigeria and *Trichophyton violaceum* in North Africa. Generally, infection outbreaks emerge due to indirect spread via external agents. For example, sharing combs or equipment which is used by hairdressers or person to person spread under overcrowded environment such as in schools or refugee camps [7].

There are few Tinea Capitis studies conducted in Ethiopia. These studies are aimed to find out Tinea Capitis was caused by dermatophytes among school-age children [8,9]. Hence up-to-date information on the prevalence of Tinea Capitis and its etiological agents is of essential. To this end, studying Tinea Capitis irrespective of age, and the spread of fungi involved in causing Tinea Capitis should be one of the preferences in health-related researches in Ethiopia.

1.2 Statement of the Problem

In the last few decades, Tinea Capitis infections have an international distribution with most cases being detected in underdeveloped countries [7]. Although this infection is considered to be a trivial disease, the psychological and emotional effects of ringworm is a burden to patients as it is a costly disease in terms of time off from work and medical expenses. In addition, the physiological trauma of this infection negatively affecting the performance and development of school children. Moreover, the increase in Tinea Capitis becomes a burden to the public health system. Tinea Capitis is predominantly caused by dermatophytes; however, non-dermatophytes Tinea Capitis fungi are increasingly implicated in causing the infection.

According to various studies in various countries, Tinea Capitis can be transmitted from infected animals to human, from soil to human and from human to human. Factors such as low social economic status, climate factors, poor personal hygiene and family history of Tinea Capitis also contributed to the transmission of Tinea Capitis in developing countries. The prevalence in children ranges from less than 1% in Western Europe to 50% in Ethiopia where the infection is endemic. In North America, the prevalence is estimated to range from 3% to 8%. It is unclear whether this increase is due to the increase in population of African immigrants to North America [10].

The magnitude of Tinea Capitis and its pathogens are different among various geographical regions. Also, it is influenced by a number of factors. The prevalence of Tinea Capitis and the predominant etiologic agents particularly of non-dermatophyte molds are poorly known in Ethiopia. The aim of this study is to assess the incidence of Tinea Capitis and the spectrum of dermatophytes and non-dermatophytes fungi implicated in causing Tinea Capitis in Ethiopia.

1.3 Significance of the Study

The results achieved in our study may be used as a reference for future epidemiological studies of this infection in Ethiopia. Assessment of the prevalence of Tinea Capitis is critical because it will provide relevant information on the spread of this infection, to develop infection prevention mechanism and to develop appropriate diagnosis strategies also knowledge of the etiology and selection of proper antibiotics for treatment.

2. Literature review

As per the past three decades studies, the prevalence of Tinea Capitis has been increasing and become one of the major health problems among children worldwide. According those studies, the incidence of Tinea Capitis has increased worldwide with an estimated prevalence of 10% in the developed nations and expectedly higher rate in most developing countries [11].

In Europe *M. canis* is widely reported causative agent of Tinea Capitis and percentages of isolation from this clinical form in Italy range from 82.7% to 90.5% [12]. Particularly *M canis*, continue to be a common cause of Tinea Capitis in many countries of the Mediterranean basin the most dermatophyte carriers being stray cats and dogs as well as pet puppies, rabbits and kittens [13].

In five villages around Eskisehir, Turkey, there was a cross sectional study conducted with a total of2384 students. Researchers establish out nine point three percent of cases have Tinea Capitis. The predominant dermatophyte isolate in this study was *T. rubrum* 43%. Their study computed using various independent variables such as older age, male, lack of hygiene, living conditions, mother's education level, previous cases of dermatophytosis and sanitary conditions associated with dermatophytosis disease [14].

A prospective, cross-sectional study to evaluate for any association between the clinical sign, microscopic and culture findings in the patients isolation and identify the frequent fungal species accountable for producing Tinea Capitis in North India among children up to 12 years of age, presenting to the Dermatology Outpatient Department between April 2006 and December 2008 with a suspected diagnosis of Tinea Capitis in214 patients with the suspected diagnosis of Tinea Capitis was found to be most frequent in the age group of 8 up to10 years, with non-inflammatory Tinea Capitis being the predominant type (56.5%). Out of culture positive cases mixed samples was recorded in 10%.From microscopic examination 41.5% cases identified an endothrix pattern of hair invasion. Simultaneously both endothrix and ectothrix pattern of invasion showed 8.8% of the cases. The predominant fungal isolated was *Trichophyton violaceum*. All the cases were examined by culture. No growth at the end of 6 weeks was recorded in 58 of the cases (27.1%). The most common isolate species *Trichophyton violaceum*

that accounting for 138 cases (88.6%). This was followed by *T. rubrum* four cases, *T. tonsurans* three cases, *M. audouinii* three cases, *T. terrestrae* two cases and six cases contaminant growth [15].

A cross sectional study, carried out by Puri *et al* in India. Among fifty patients from the dermatology department the preschool going children were selected for the study. T. Capitis in India were evaluated for clinical presentation, age and sex distribution, Family history of Tinea Capitis and type of fungi isolated from the lesions. There were 15 females and 35 males between 3 to 10 years of age. Tinea Capitis and 32% patients showed non-inflammatory Tinea Capitis. Family history of Tinea Capitis which was seen in 29% of patients may be due to sharing of articles by other family members65% patients were recovered on culture positive and regarding the causative agent of fungi no growth was seen in 20% patients. The lower middle of the children belonged to 82% and the lower income groups belonged to 22%. Culture specimens from all the cases were examined. No growth of fungi was recorded in 10 of the cases (20%). fungal elements growth, *T. violaceum* was the most frequent isolate in 37 cases (74%). This was followed by *T. rubrum* (one case), *T. tonsurans* (two cases), *M. audouinii* (two cases)[16].

Study conducted in India. The incident and etiological agent of dermatophytosis in the region of Tiruchirapalli. For a year, among a total of five hundred nineteen samples were received from hair, skin and nails of the patients. This study showed that more dermatophytosis cases most frequent in age group of 11 to 20 years and 21 to 30 years. The test result showed that Tinea corporis the most common that accounted 35.4%and tinea cruris that accounted 16.8% and Tinea Capitis also accounted16.7%; Tinea Capitis was predominant in children which is below twelve years; boys were affected more than the girls 67.1%; dermatophytes occurrence in seventy percent of the samples and the most common etiological agent was *T. rubrum* was followed by *T. mentagrophyte* [17].

In a prospective study conducted in Department of Dermatology of SKIMS-MCH, Srinagar, Jammu and Kashmir, India from April 2015 to March 2016, out of 457 cases of Tinea Capitis14 patients were adults and represented 3.06% of all cases. All patients were females. The most

common isolated etiological agent was *T. violaceum* (35%), followed by *T. mentagrophytes* (21.43%), *T. tonsurans* (14.29%), *T. rubrum* (14.29%) and *T. schoenleinii* (14.29%) [18].
A cross sectional in Iraq among the 56 children with Tinea Capitis was positive in 89.2% of the 56 cases of Tinea Capitis. Which is isolates of *M. canis* (35/62.5%), *T. tonsurans* (15/26.8%), *T. mentagrophytes* (4/7.1%) and *T. rubrum* (1/1.8%). Among the 79 adolescents, adults and elderly living with children with Tinea Capitis 2.5% (2 cases) were asymptomatic carriers (confidence interval 95% [0.3-8.8%]) and 3.8% (3 cases) had the disease (confidence interval 95% [1.0-11.4%]) [19].

From 112 skin infections were investigated in Saudi Arabia, in this study the researchers finds out Tinea Capitis infection was the most common infection among the patients. The result of this study shows that 52 dermatophyte isolates and identified. The isolates dermatophytes which were nine species such as *T. concentricum, T.violaceum, , T. rubrum, T. verrucosum, T. schoenleinii, T. mentagrophytes , E. floccosum, M. canis* and *M. audouinii* those are isolated etiological agent that cause skin infections. The most common isolated etiological agent was *M. canis*. Non dermatophyte isolates included five cases isolates from *Aspergillus spp.* four isolates from *Acremonium potronii* and fifteen isolates from *Candida spp*[20].

A cross-sectional study done by Adefemi *et a*l. the study finds out the clinical sign, prevalence in addition to the causative agents was conducted in Nigeria. Among 602 children with in the age range 5 to16 include in this study. The study confirms that **t**he prevalence of clinically sign dermatophytosis lesion was 29.9%, in those children the predominant isolated was non-dermatophyte fungi that account for 15.4%, followed by 5.0% dermatophyte isolated. Tinea Capitis was the predominant infection, then Tinea Corpores and Tinea pedis; mixed infections have observed in nine cases. Also, in this study identified only three species of dermatophytes were accountable for human infection in the area study site. The commonest isolated etiological agent was *T. mentagrophyte* followed by *M. audouinii* and *T. verucossum*[21].

The study that was conducted in Nigeria in 2016 shows that the diagnosis of Tinea Capitis has been increasing. The researchers screened 100 school children, prepared fungal culture and microscopy those children. The result showed that 45% of the children were diagnosed with

Tinea Capitis. Among the various dermatophytes that were isolated, *Trichophyton rubrum* (28.8%), *Microsporumcanis*(22.7%) *Trichophyton verrucosum* (4.5%) and *Trichophyton tonsurans* (4.5%) were the most prevalent dermatophytes. According to the study, there were pure infections that accounted for 73.3% although 26.7% had multiple infection with two up to four dermatophytes of the genera *Trichophyton* and *Microsporum* [22].

Similarly, and more expanded study involving several states in Central Nigeria, a total of 28,505 primary school children aged between 3 and 16 years were sampled from 12 primary schools. Tinea Capitis was more common in males 78.2% than females. The predominant infected age group 10-14 years (42.7%) followed by5-9 (40.3%). There was significant correlation between occurrences of Tinea Capitis and age group. The prevalence of Tinea Capitis was influenced by cultural and social practice of the areas. *T. soudanense* was most common etiological agent isolated for this study, followed by *M. ferrugineum* and *M. audouinii* [23].

An investigative study was conducted among Islamiyya school children, age group of 5 to 13 years in Nigeria. This study examined the incidence of dermatophytosis and associated non-dermatophytes from a total sample of 100 cases. The study recorded *M. ferrugineum* (15.4%), *M.canis*(15.4%), *M. audounii* (9.9%) and *T. concentricum* were the dominant representative species of dermatophytes. Lower frequencies of dermatophytic and non- dermatophytic fungi were observed to be consistent with children who had school chairs compared to those that sat on mats and on bare classroom floor respectively. The total number of 30 children who played only at school recorded 26.4% (24) fungi isolates (dermatophytic and non-dermatophytic) while those who played both at school and home respectively showed high incidence of 73.6% (67) of fungal isolate. The distribution of fungal isolates among children who played with domestic dogs, cats, goats and poultry birds were 13 (14.3%), 35 (38.5%), 22 (24.2%) and 13 (14.3%) respectively. On the contrary, a frequency value of 8.8% was recorded for the population of children that had little or no contact with any form of domestic animal [24].

Similarly, in a study conducted in the outpatient dermatology clinic, BeniSuef University Hospitals, in Egypt during a 3-month period, from February 2016 to April 2016 among 100 children (85 male and 15 female) aging from 1 to 16 years and diagnosed clinically with Tinea

Capitis. The majority of this study 97% showed gray patch lesion, while only 3% had kerion. In addition, 38% of the patients had single lesion, while 62% patients had multiple lesions. Per this study, 85% of the microscopic and culture tests were positive by direct microscopy while 92% had positive culture. Approximately,79% of the samples were positive for both direct microscopy and culture, while only 4% were negative for both. *M. canis* was greatly isolated organism in almost two-thirds of the patients (64%) followed by *T. mentagrophytes* (16.3%), while *M. audouinii* was the least frequently isolated organism [25].

Researchers randomly selected students from Mathare, Nairobi, five public primary schools going children to conduct a cross sectional study. This study was conducted from May to September 2013 among children age group between 4 and 14 years. In this study, 89 (59%) males and 61 (41%) females were enrolled. A total sample of 150 children examined. Of those,122 (81.3%)samples were diagnosed with fungal lesions caused by one of the three dermatophytes. *Trichophyton* were accounted 61.3% cases, followed by*Microsporum*13.3% cases and *Epidermophyton* 7.3% cases. In addition, 102 (68%) were symptomatic while 48 (32%) being asymptomatic cases. Although all cases which is signs of the infection were identify positive, only 43.75%cases showing no symptom were identify positive for Tinea infections. This study show that boys (45.3%) were mainly infected compared to girls (36.7%) with gender being a significant risk factor to infection. Researchers found out that the most prevalent dermatophyte isolated was the anthropophilic dermatophyte*T. rubrum* [26].

A study conducted in Tulugudu Island, Ethiopia. The researchers screened 171 children age group between 4 and 15 years. The result showed that above 90% of the children were shared combs bed, had animal contact and also family size was greater than five people. In fifty percent of the test subjects with this group prevalence of Tinea Capitis was elevated. In this study, 85 (49.7%) males and 86 (50.3%) were females. Among screened children most of them microscopy-positive 74.1% and culture positive for dermatophytes that accounted for73% The prevalence of dermatophytes was shown in children 57.3%cases. Tinea Capitis was the highest manifestation in 76.5% cases. The study found out that *T. violaceum* in 80.6% cases were the leading species followed by *T. verrucosum* 16.3% cases and *T .tonsurans was in* 2% cases [27].

A cross-sectional study conducted in Ethiopia, Harari Regional State, Ethiopia to carried out the prevalence and causative agents of dermatophyte infections. The study in this region revealed. The study in this region revealed that out of the 428 school children from nine different primary schools. Most of those school children 67% werein the age range of 10 to 14 years. Out of this, 211 (49%) were males and 217 (51%) were females.Out of, 100 (23.4%) had culture positive for dermatophytosis and the frequency of Tinea Capitis that accounted for 18% (77/428)..The predominant isolated etiological agent was *T.violaceum* that accounted for 38.8%; followed by *T. rubrum* accounted for22.4%.while in rural residents the predominant isolated species was *M. canis* (37.5%).The main incidence of dermatophytosis was shown in this study. Therefore, the study found out that prevalence of dermatophytosis in the study site school children and Tinea Capitis was the most common clinical finding which needs an involvement [28].

2.1Conceptual frame work

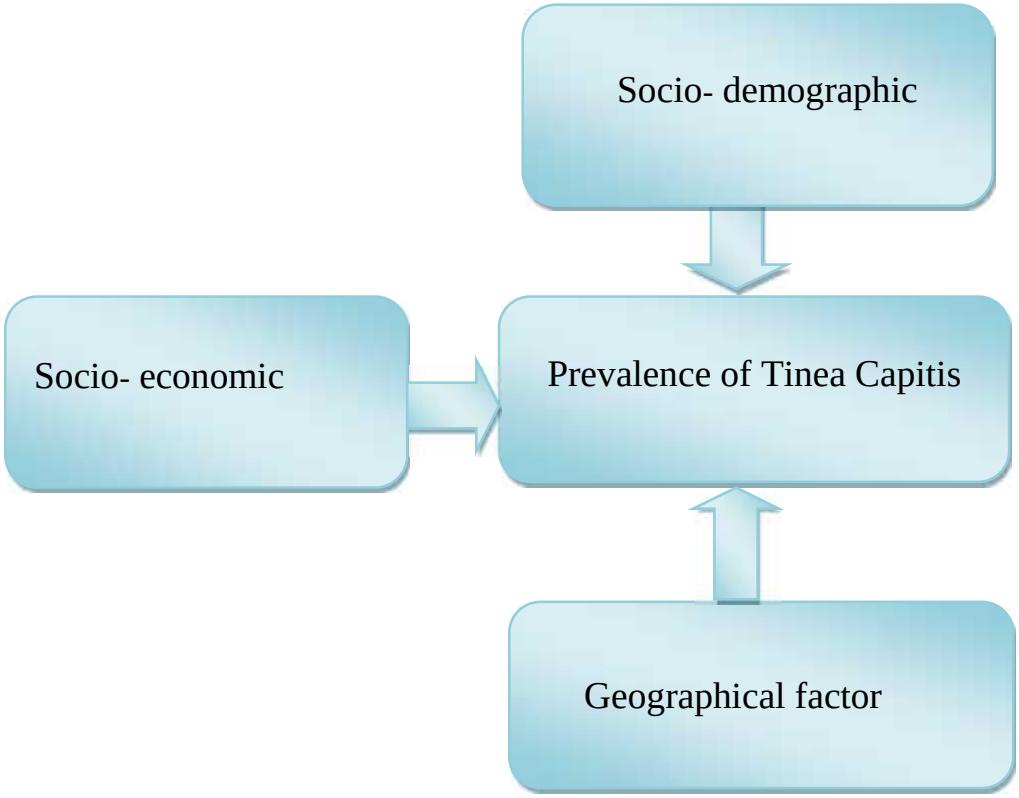

Figure 1: Conceptual framework

3. Objectives

3.1. General objective

Our study aimed to find out the prevalence of Tinea Capitis and the mycological profile of dermatophytes and non-dermatophytes fungi implicated in causing Tinea Capitis among patients from at Rank Higher Specialized Dermatology Clinic, Addis Ababa, Ethiopia.

3.2. Specific objectives

- ✓ To determine the prevalence of Tinea Capitis.
- ✓ To determine the prevalence of Tinea Capitis among different socio-demographic characteristics.
- ✓ To determine the prevalence of dermatophytes and non-dermatophytes implicated in causing Tinea Capitis.

4. Hypothesis

The prevalence of dermatophyte and non-dermatophyte was very common for patients treated at Rank Higher Specialized Dermatology Clinic.

5 Methods and Material

5.1 Study area

Our study was carried out at Rank Specialized Dermatology Clinic found in Kirkos Kefle Ketema, Sub City Wollo Sefer, Addis Ababa, Ethiopia. Rank Specialized Dermatology Clinic Family PLC is a company established in 2005 in Addis Ababa Ethiopia. The first company was established as Rank Medium Clinic which was upgraded after two years as Rank Specialized Clinic. Then it was established as a medium general clinic in 2005. It was upgraded to a specialized dermatology clinic from 2008 onwards. In these 8 years, the clinic has achieved to have a wide dependable customer base from all over the country including Addis Ababa. The clinic has several specialists. There are five experienced dermatologists working in the clinic. There is one part-time surgeon. There are ten nurses working in this clinic and three of them are trained in aesthetic care. There are three lab technicians working in the lab The Clinic has five experienced dermatologists, one part-time surgeon, seven nurses, three aesthetic care nurses and three lab technicians. The Clinic has a laboratory which is used by five departments including mycology. This lab has an average turnover of 15 to 20 mycological suspect cases per day. This Clinic has several organizations as credit customers including different banks insurances embassies and hotels.

5.2 Study Design and Period

A prospective non-randomized study was conducted at Rank Higher Specialized Dermatology Clinic fromFebruary2019-July2019.

5.3Population

5.3.1 Source Population

Our population sizes were patients who were attending Rank Specialized Dermatology Clinic.

5.3.2. Study population

Clinically suspected patients with Tinea Capitis infection and referred to the laboratory at Rank Specialized Dermatology Clinic.

5.4 Inclusion and Exclusion Criteria

5.4.1. Inclusion Criteria

Patients who were clinically suspected of Tinea Capitis infection at Rank Dermatology Clinic were included during the study period.

5.4.2. Exclusion Criteria

Patients under fungal treatment were excluded.

5.5. Study variables

5.5.1. Dependent variables

- ✓ Prevalence of Tinea Capitis.
- ✓ Spectrum of dermatophytes and non-dermatophytes fungi.

5.5.2. Independent variables

- ✓ Socio-demographic characteristics such as: age, gender.

5.6 Measurement and Data Collection

5.6.1. Sample Size calculation

The sample size has been determined by taking the prevalence of Tinea Capitis (18.0%) from previous study conducted in Ethiopia [28] using the formula for single population proportion. The sample size for single population proportion was calculated using the following formula

Significance level calculated at 95%CI

Margin of error tolerated is 5 % (0.05)

$$n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d2}$$

Where: n is n minimum required sample

Z is Z-score at 95% CI (1.96)

P is population proportion (18 %)

d is margins of error (0.05)

$$n_0 = \frac{(1.96)^2 (0.18)(1-0.18)}{(0.05)^2}$$

$$= \frac{(3.84)(0.18)(0.82)}{0.0025}$$

$N_0 = 227$

5.6.2 Sampling method

Convenience sampling technique was applied to recruit study participants which clinically suspected cases of Tinea Capitis infection prescribed by physicians included. Therefore, the final total sample size required for this study after considering a 10% contingency was 250.

5.6.3 Data Collection Procedure

5.6.3.1Demographic data

The socio-demographic data was collected by questioner. If patients were minor, their guardians were asked to complete the form. Study participants obtained the form from the laboratory. The patient assent and consent forms were filled during sample collection. Before the actual data who collection, a pre-test on calculated sample size for pre- test was conducted using demographic and clinical data collection formats and log books. The purpose of the study as well as any related harm and benefit were explained to the study participants accordingly.

5.6.3.2 Specimen collection and transportation

The specimens were collected for microbiological analysis from clinical diagnosed patients with scalp infections fungal origin by dermatologists. In order to remove any oil from the patient's scalp, we requested them to come after hair wash. For all patients, we wiped the infection region of a skin with 70% alcohol and allow the alcohol to evaporate. Then scalp scraping and hair fragments collected from active border of the lesion using sterile blades. Specimens were divided by two parts. The first part was used for microscope examination. And the second part was transported the sample properly labeled in date of collection and sample code on sterile plastic petri dishes to college of health science, Department of Laboratory Science, Addis Ababa University for further analysis. As a procedure, before clinical samples were collected, patients who were older than 18 years old were required to complete and signed consent forms. If patients were less than 18 years old (minor), the form had to be fulfilled and signed by the parents or guardians.

5.6.4Laboratory Analysis

5.6.4.1 Detection, isolation and characterization fungal pathogens

Direct microscopy (KOH)

A small amount of scalp scraped sample was placed on a cleaned slide and added one drop of 10% potassium hydroxide solution. After 5 minutes, the wet mount was analyzed under a low power filed of microscope for the presence of fungal elements spore size and appearance inside (endothrix) or outside (ectothrix), the hair shaft distinguishes organisms. (Annex I)

Culture isolation of fungal pathogens

All clinical samples were cultured regardless of the positive or negative direct microscopic examination results. All clinical sample were cultured by streaking on two plates of Sabouraud's dextrose agar (SDA) without cycloheximide and Mycosal agar containing with chloramphenicol containing antimicrobial antibiotics inhibits the growth of bacterial(Oxoid, Basingstoke, England).Gentamicin is added to further inhibit the growth of gram-negative bacteria. The culture media were prepared according to the manufacture's procedure. All inoculated plates culture is sealed with plastic tape and incubated inverted position at 25^{o}C for molds & 37^{o}C for yeasts & keep up to four to six weeks if no growth. Incubated plates were observed after three days for fungal growth. When we suspected colonies of molds we used potato dextrose agar for sub cultured in to identification for slow growth of molds. (Oxoid, Basingstoke, England).To makes ready to use the media according to the manufacture's instruction. Mold isolates were identified by examining macroscopic identification and microscopic characteristics (i.e., texture, rate of growth, topography, and pigmentation) of their colony. Then we prepared to examined for microscopic identification of mold isolates was performed by placing pieces cultures from SDA to clean microscopic slide and staining with lacto phenol cotton blue solution on a glass slide covered with cover slide. After that we were observed microscopically [29]. Mycological laboratory books and manuals were used as reference resources in the process of identification [30-31]. A few physiological tests were done to aid in species differentiation within a given genus since some share same characteristics. They include Urea hydrolysis for *Trichophyton equinum* which doesn't hydrolyses urea.

5.7 Data Quality Assurance

Data quality was ensured through the use of standardized data collection materials, and pre-test was done before regular data collection. This provides information on fungal media, including application, selection, inoculation and schedules for reading Media were checked for sterility and growth supporting ability and standard protocols were followed. Questionnaires used to collect socio demographic data but incomplete forms were discarded. The specimen collection container was clearly labeled (unique sample code, date of collection) and transported to for further analysis. Before we used all equipment were checked for their functionality. Before using the prepared culture media each batch were checked for sterility by incubating the portion of prepared media in petri dish checked for waiting overnight and observe for the presence of any growth, unsmooth distribution media and if any color change of the media it was be discarded and re-prepared the media. We were using the control strains before we performed culture.

5.7.1 Quality Control

5.7.1.1 Pre- analytical

The patients were asked to come after hair wash so as to remove any oil from the scalp. For all the patients, we wiped the infection region of a skin with 70% alcohol and allow the alcohol to evaporate.Then removed epidermal scales at the active border of the lesion with scalpel. Reagents used for KOH and SDA media preparation were checked for expiry date and any abnormal color change. Preventive maintenance of equipment was inspected.

5.7.1.2 Analytical

- ✓ Media preparation was performed according to manufacturers' manual.
- ✓ Test procedures for each test were strictly followed according to standard operation system of the tests.
- ✓ Prepared SDA, medias were checked for growth support by incubating at 25-30^{o}C for weeks.
- ✓ Sterility of each batch of prepared media was checked by incubating un inoculated media at 25-30^{o} C for weeks.
- ✓ All laboratory specimens were treated as infectious and handled according to standard precautions the specimen was processes on bio safety cabinet class II.

5.8 Data analysis and interpretation

Data coded entry and analyses were done using Statistical Package for Social Sciences (SPSS) software version 23.Descriptive statistics were used to explanatory for study participants used to highlight potential relationships between the study participants and significant variables such as socio demographical characteristics (age, sex). Descriptive statistics which gave us frequency and percentage of the data. Chi-square test was used to test associations between the prevalence of the Tinea Capitis infection by gender and age group. P value regard as significant if less than0.05. Finally, the results were presented on words, graphs and tables.

5.9 Ethical considerations

This study was carried out after getting ethical clearance and formal letter of cooperation obtained from the Ethics Committee of the Departmental Research and Ethics Review Committee of Addis Ababa University, College of Health Sciences, department of laboratory to conduct the study The letter was taken to Rank Higher Specialized Dermatology Clinic and permission was obtained from Rank Higher Specialized Dermatology Clinic. All the necessary precautions were taken to keep all documents in a safe and secure place. Names and other identification of the study subjects were omitted during data collection. Each participant was informed briefly about the study. Each interviewer was given an opportunity not to take part in this study and to change their mind during the study period. Those showing interest to be included and willing to give consent were only included in the study. If the diagnosis showed a positive test for fungal pathogen, the participants were informed by the Clinic's Clinician. After the information was read for study participants, an assent form was completed and signed by the study participants. If the participants are under age (under 18 years of age), a family member and/or adult guardian should complete and signed the forms.

5.10 Dissemination of Results

The final result of the study was submitted and presented to Addis Ababa University College of Health Sciences, Department of Laboratory Sciences. In addition, the research will be presented different seminars, workshops and conference and it will be submitted to pre reviewed journals for publication.

6. Results

6.1 Demographic analysis

The participants in this study attended at Rank Higher Specialized Dermatology Clinic in Addis Ababa, Ethiopia and a total of 364 samples which was collected by scraping scalp from the individuals suspected with Tinea Capitis. The study comprises both genders and wide age ranges from one to sixty-four. As shown in Table-1 below, more female than males. Out of the total samples, 228 are females and 136 are males. The percentage distribution between females and males are 62.6% and 37.4% respectively with the ratio of 1.67:1. Out of the classified age groups, the highest number was observed in the age group of 1 to 14 years with total number of 280 which is 76.9%.

Table1: Study participants samples by gender and age group

Age Group	Female	Male	Total	Percentage by Age Group
1 - 14	169	111	280	76.9
15 - 24	16	3	19	5.2
25 - 44	37	18	55	15.1
45 – 64	6	4	10	2.8
Total	**228**	**136**	**364**	**100**

6.2 Prevalence of Tinea Capitis

The result of the laboratory test revealed that 301 (82.7%), out of 364 study participants, found fungal positive. Further analyzing the incidence of Tinea Capitis in different age groups uncovered that the age group 1 to 14 years were the most commonly affected ones than the other age groups. As shown in the Table – 2 below, the analysis showed that there was high significant correlation between age group and fungal infections (P=0.000). Also, out of 301 fungal positive cases 183(60.7%) were females and 118 (39.3%) were male. However, no association was found between gender and fungal isolates (P=0.105).

Table 2: Prevalence of Tinea Capitis study participant samples by gender and age group

Variables4	Categories	Number of Samples (%) with Tinea Capitis	Total Number of Samples (%) processed	P-Value
Age Groups	1 - 14	254 (84.4)	280 (77)	0.000**
	15 – 24	10 (3.4)	19 (5.2)	
	25 – 44	30 (10)	55 (15)	
	45 – 64	7 (2.3)	10 (2.8)	
Gender	Female	183(60.7)	228(62.6)	0.105
	Male	118 (39.3%)	136 37.4)	

Note: According to WHO age classifications for health 2007, **P statistically high significance

From364 samples, 301 (82.7 %) were found to be fungal positive by culture for both dermatophyte and non-dermatophyte fungi microbiologically, while 225 (61.8) were direct microscope positive. There are some incidents recorded that the result came out positive in direct examination but negative in culture and vice versa. As an example, in this study 11 cases were observed positive in direct examination but found negative in culture and 87 cases was observed negative in direct examination but fungi were grown in culture. Clinical analysis also demonstrated that out of 364 samples 214 (58.7%) came out positive in both culture and direct microscopic examination. Out of 364 study samples 52 (14.4%) of the samples were free from fungi detection nor growth. Table -3 below summarizes the outcome of microscopy test results based different test procedures.

Table 3: Correlation of direct microscopy with culture

Test procedure	Number	Percentage
Direct microscopy positive	225	61.8
Culture positive	301	82.7
Direct microscopy positive culture positive	214	58.7
Direct microscopy positive culture negative	11	3
Direct microscopy negative culture positive	87	23.9
Direct microscopy negative culture negative	52	14.4

6.3 Fungal pathogens group

The clinical examination further identified the cause of fungal infections. Among the group of fungal etiology belonging to dermatophyte, non-dermatophyte and mixed. Out of 301 cultures positive patients, these three species of fungal infections were further isolated and identified. Dermatophytes were high 136 (45.2%) followed by non-dermatophyte molds 121 (40.2%) and mixed infections 44(14.6%) were isolated (figure 2).

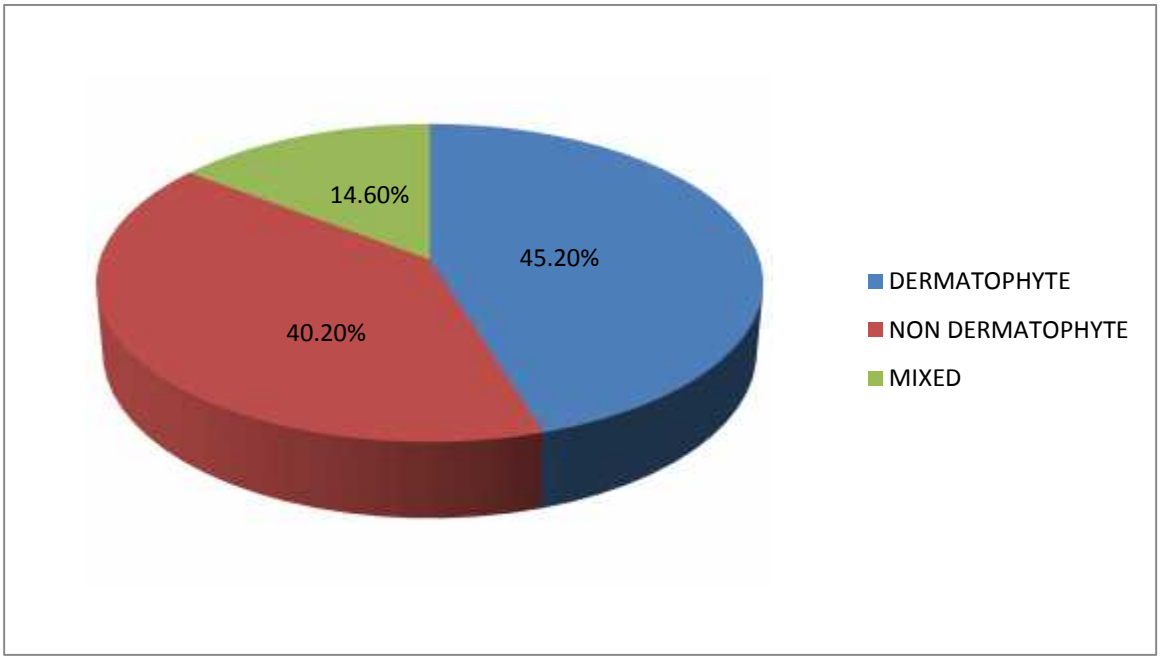

Figure 2: proportion of fungal groups isolated from study participant with Tinea Capitis.

Table4: Spectrum of fungal isolated from study participants.

Fungal species	Single isolates	Mixed with other fungi	Total isolates
Dermatophytes			
Trichophyton violaceum	117	13	130
Trichophyton mentagrophytes	8	7	15
Trichophyton tonsurans	4	8	12
Trichophyton verrucosum	3	3	6
Microsporumaudouinii	4	3	7
Dermatophytes Sub-total	**136**	**38**	**170**
Non- dermatophytes			
Acremonium Spp	1	-	1
Alternaria Spp	5	3	8
Aspergillus flavus	2	1	3
Aspergillus fumigatus	69	6	75
Aspergillus niger	4	2	6
Aspergillus Sp	1	3	4
Aspergillus terreus	1	-	1
Bipolaris	2	-	2
Cladosporium Spp	14	7	21
Curvularia Spp	1	-	1
Epicoccum purpurascens	1	-	1
Exophiala jeanselmei	2	-	2
Exserohilum Sp	1	-	1
Fonsecaeapedrosi	1	1	2
Fusarium Spp	5	5	10
Mucor	1	-	1
Penicillium	5	2	7
Rhizopus	3	-	3
Scopulariopsis Sp	2	-	2
Non-dermatophytes Sub-total	**121**	**30**	**151**
Trichophyton violaceum+Aspergillus fumigatus	-	5	5
Trichophyton violaceum+Aspergillus Sp+penicillium	-	1	1
Trichophyton violaceum+Aspergillus Sp+Aureobasidium Sp	-	1	1
Trichophyton violaceum+Cladosporium Spp	-	2	2
Trichophyton verrucosum+Cladosporium Sp	-	2	2
Trichophyton verrucosum +Phialophora	-	1	1
Trichophyton verrucosum+Trichophytontonsurans+Cladosporium Sp	-	1	1
Cladosporium Sp + Trichophyton mentagrophytes	-	1	1
Cladosporium Sp+ Trichophyton violaceum+ Trichophyton soudanense	-	1	1
Trichophyton mentagrophytes	-	1	1

+Scedosporium+Phialophora			
Microsporum audouinii +FusariumSp	-	1	1
Cladosporium Sp+ Trichophyton violaceum +Fusarium Sp	-	1	1
Trichophyton mentagrophytes +Scytalidium dimidatium	-	1	1
CladosporiumSp+ Trichophyton violaceum	-	1	1
Trichophyton mentagrophytes +Aspergillus flavus	-	2	2
Trichophyton mentagrophytes+ Trichophyton tonsurans	-	3	3
Trichophyton mentagrophytes +Microsporum audouinii	-	1	1
Trichophyton tonsurans++ Trichophyton violaceum	-	1	1
Alternaria Sp+ Trichophyton schoenleinii	-	1	1
AlternariaSp + Trichophyton violaceum	-	2	2
AlternariaSp+ Trichophyton violaceum +Fusarium Sp	-	1	1
Aspergillus fumigatus + Microsporum audouinii	-	1	1
Trichophyton mentagrophytes +Fonsecaea Pedrosoi	-	1	1
Trichophyton violaceum +Aspergillus niger	-	2	2
Fusarium Sp +Ulocladium Spp	-	1	1
Trichophyton tonsurans +Aspergillus Sp	-	2	2
Trichophyton tonsurans +pencillium Sp	-	1	1
Fusarium Sp+ Trichophytonviolaceum	-	1	1
Trichophyton tonsurans+ Trichophyton violaceum	-	2	2
Trichophyton tonsurans +Aspergillus flavus	-	1	1
Trichophyton tonsurans+Microsporum audouinii	-	1	1
Total no. samples with mixed culture	-	**44**	**44**

As shown in Table 4, the fungal species were categorized as Dermatophytes and Non-Dermatophytes. Out of the 364 total samples collected from the suspected patients, dermatophytosis, fungi were isolated in 301(82.7%) of patients. In comparison, dermatophytes were the most common isolated 136 (45.2%) than non-dermatophytes molds 121 (40.2%). More than one fungal isolated were detected in the remaining 44 (146%) cases out of 301 positive samples. Out of 136Single (pure) isolates dermatophytes *T.violaceum* was the most common pathogen accounted for 117 (86.02%) followed by *T. mentagrophytes* that accounted 8 (2.2%) cases. Both *T .tonsuransi and M. audouinii* show 4 (2.94%) cases. The least isolated cases were *T.verrucosum* 3(2.2%).

Out of those 121(40.2%)Single (pure) isolates non-dermatophytes molds, the highest number were *Aspergillus fumigates* that accounted for 69 (57.02%) followed by *Cladosporium Sp* that accounted for 14(11.57%). The remaining isolated *Acremonium Spp., Aspergillus terreus, Aspergillus Spp., Curvularia Spp., Epicoccum purpurascens, Exophiala jeanselmei, Fonsecae apedrosi* and *Mucor* were accounted for only 1(0.82%) case. *Alternaria Spp., Fusarium Spp.* and *Penicillium* were accounted for 5(4.13%) cases. *Aspergillus niger* was accounted for on 4 (3.30%) cases. *RhizopusSpp.*were accounted for 3 (2.47%) cases. *Aspergillus flavus, Bipolaris, Exserohilum Spp.* and *Scopulariopsis Spp.* were accounted for on 2(1.65%) cases as shown in Table 4.

In this study, among 301 fungal positive cases, both dermatophyte and non-dermatophyte infections were isolated in 44(14.6 %) patients of Out of those 44 mixed samples, 37 fungal isolated with two different species. In 7 cases, we found triple different species with dermatophyte and non-dermatophyte agents. Also, those species only found mixed with other fungi for non-dermatophyte *Aureobasidium Spp. Phialophora, Scedosporium, Scytalidium dimidatium* and *Ulocladium Sp.*For dermatophyte *Trichophyton soudanense and Trichophyton schoenleinii*.

Table5: Spectrum of fungal pathogens in age group among study participants

Isolated agents	Age Group				Total
	1-14	15-24	25-44	45-64	
Acremonium Sp	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (0.3%)
Alternaria Sp	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.7%)
Aspergillus flavus	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	2 (0.6%)
Aspergillus fumigates	53 (76.8%)	3 (4.3%)	9 (13.0%)	4 (5.8%)	69 (22.9%)
Aspergillus niger	3 (75.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	4 (1.3%)
Aspergillus Sp	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)
Aspergillus terreus	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)
Bipolaris	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	2 (0.6%)
Cladosporium Sp	11 (78.6%)	1 (7.1%)	1 (7.1%)	1 (7.1%)	14 (4.6%)
CurvulariaSp	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (0.3%)
Epicoccum purpurascens	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.3%)
Exophialajeanselmei	1 (50.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	2 (0.6%)
ExserohilumSp	0 (0.0%)	0 (0. 0%)	1 (100.0%)	0 (0.0%)	1 (0.3%)
Fonsecaeapedrosi	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	1 (0.3%)
Fusarium Sp	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.6%)
Microsporumaudouinii	3 (75.0%)	0 (0.0%)	1 (25.0%)	0 (0. 0%)	4 (1.3%)
Mixed	34 (77.3%)	4 (9.1%)	6 (13.6%)	0 (0.0%)	44 (14.6%)
Mucor	1	0	0	0	1 (0.3%)

	(100. 0%)	(0.0%)	(0.0%)	(0.0%)	
Penicillium	5 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (1.7%)
Rhizopus	3 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (10%)
ScopulariopsisSp	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.6%)
Trichophyton mentagrophytes	8 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (2.6%)
Trichophyton tonsurans	4 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (1.3%)
Trichophyton verrucosum	2 (66.7%)	0 (0.0%)	1 (33.3%)	0 (0.0%)	3 (10%)
Trichophyton violaceum	110 (94.0%)	1 (0.8%)	6 (5.2%)	0 (0.0%)	117 (39.0%)
Total	254 (84.4%)	10 (3.3%)	30 (10.0%)	7 (2.3%)	301 (100 .0%)

The study also categorized fungal pathogens based on different age groups. The highest fungal pathogens or dermatophytosis were in age group of 1 to 14 years. The highest frequency of the isolated agents from dermatophyte recorded in this age category most common species was *Trichophyton violaceum* 110 (94%) followed by *Aspergillus fumigates*53 (76.8%). In this age category, the following isolated agents were not detected in *Acremonium Sp,Aspergillus terreus*, *Curvularia Sp*, *Exserohilum Sp* and *Fonsecaea pedrosi.* As shown in Table 5, the highest fungal pathogens in the overall age group were *Trichophyton violaceum* 117 (39.0%) which was followed by the next highest non-dermatophyte species was *Aspergillus fumigates* 69 (22.9%). The next age group that was affected by dermatophytosis was 25 to 44 years. The only species that was isolated in this age group were (*Acremonium Sp, Curvularia Sp, Exserohilum Sp* and *Fonsecaea pedrosi*). The list affected age group was 45 to 64. The frequency in this age group accounted 7 (2.3%).Mixed infections affected mostly an age group of 1 to 14 years 34(77.2%).

Table 6: Distribution of fungal isolates with gender among study participant

Fungal agent isolates	Sex				Total	
	Female		Male			
	Frequency	%	Frequency	%	Frequency	%
Acremonium Spp	1	100.0	0	0.0	1	0.3
Alternaria Spp	2	40.0	3	60.0	5	1.7
Aspergillus flavus	2	100.0	0	0.0	2	0.7
Aspergillus fumigates	48	69.6	21	34.4	69	22.9
Aspergillus niger	1	25.0	3	75.0	4	1.3
Aspergillus Spp	1	100.0	0	0.0	1	0.3
Aspergillus terreus	1	100.0	0	0.0	1	0.3
Bipolaris	0	0.0	2	100.0	2	0.7
Cladosporium Spp	9	64.3	5	35.7	14	4.6
Curvularia Spp	1	100.0	0	0.0	1	0.3
Epicoccum purpurascens	0	0.0	1	100.0	1	0.3
Exophiala jeanselmei	2	100.0	0	0.0	2	0.7
Exserohilum Sp	0	0.0	1	100.0	1	0.3
Fonsecaea pedrosi	1	100.0	0	0.0	1	0.3
Fusarium Sp	3	60.0	2	40.0	5	1.7
Microsporumaudouinii	3	75.0	1	25.0	4	1.3
Mixed	26	59.0	18	41.0	44	14.6
Mucor	0	0.0	1	100.0	1	0.3
Penicillium	2	40.0	3	60.0	5	1.7
Rhizopus	1	33.3	2	66.7	3	1.0
Scopulariopsis Sp	1	50.0	1	50.0	2	0.7
Trichophyton mentagrophytes	4	50.0	4	50.0	8	2.6
Trichophyton tonsurans	2	50.0	2	50.0	4	1.3
Trichophyton verrucosum	1	33.3	2	66.7	3	1.0
Trichophyton violaceum	71	60.7	46	39.3	117	38.9
Total	183	60.8	118	39.2	301	100

The study further investigated the distribution of fungal pathogens based on genders. As shown in Table 6, higher fungal groups detected in female than in males. The analysis also found that *Trichophyton violaceum* fungal agent isolated are the most common ones in both females and men. There are some fungal species which is common only in females and not detected in males and vice versa. As an example, *Acremonium Sp, Aspergillus Sp, Aspergillus terreus, Curvularia Sp, Fonsecaea pedrosi, Exophiala jeanselmei* were found only in female patients and *Bipolaris ,Epicoccum purpurascens, Exserohilum Sp* and *Mucor* were found only in male patients. Also, there were some fungal species equally affected both females and males. For example, the examination demonstrated that *Scopulariopsis Sp,Trichophyton mentagrophytes* and *Trichophyton tonsurans* were found on both females and males. Out of those 44 mixed samples, 26(59%) was in female patients and 18 (41%) was in male patients.

7. Discussion

The main objective of our study was an attempt to determine the incidence and causative agents of dermatophytes and non-dermatophyte fungi collected from patients' scalps

Tinea Capitis is a common fungi infection recorded throughout the world especially, in underdeveloped countries. This study supports that the frequency of dermatophyte 136 (45.2%), non-dermatophyte 121 (40.2%) and mixed fungi were and 44 (14.6%).The overall prevalence was 82.7%. This study is similar with a study conducted in Mathare, an informal settlement in Nairobi, Kenya by Moto *et al.* (81.2%) [26], local studies Teklebirhan and Bitew reported that prevalence of dermatophyte 130(42.6%) and non-dermatophyte 94 (30.8%) fungi with the overall prevalence of 73.4% [32]. Also, Tulugudu Island, Ethiopia study showed that the over all prevalence of (79.5%) [27]. The prevalence of Tinea Capitis differs according to weather conditions, standard of living, poor hygiene, sharing of fomites, overcrowding, low socio economic, as well as the natural reservoir of infection and other factors that predisposes population to infection [12, 5].

In this study, a total sample of 364 scalp scraped were collected. Out of this sample, 301 (82.7%) clinical samples were cultured positive and 225 (61.8%) were direct microscope positive. Of those 364 samples, 214(59%) were both direct microscope and culture positive. This result is different from Arora *et al.* because their study showed that out of 657 clinical samples received, 189 (28.76%) clinical samples were direct microscope positive 91 (13.85%) were culture positive and 62 clinical samples were both direct microscope and culture positive. Also, they found out that patients presenting with a clinical finding of Tinea that had culture result was positive for infection were 13.85% [33]. In contrast, our study showed comparatively high culture positive rate (82.7%) was achieved. From analysis of our data detection of fungus by microscopic examination is lower compared to culture method. Their reasoning was that their culture results got small percentage of miss diagnosis of Tinea including sampling error, using out of order culture media and mismanaging of the culture media and mishandling of the samples. Therefore, culture can be used as a definitive procedure for screening and diagnosis of dermatophytic infection. It is essential that good laboratory methods should be available for precise identification of the dermatophytes [34].

Our result showed that dermatophytosis was common (76.9%) in the age group of 1 to 14 years. There was high significant association between age group and fungal infection which gave us P=0. 000. Whereas age group above 44 years had the lowest 2.7%. This result closely related with other study conducted in Ethiopia. Out of 153 patients with Tinea Capitis, 73.2% were in this age group [35]. Many concurrent studies showed that the most frequent scalp infection affected the primary school children [22–27]. This is because of poor personal hygiene at this age as well as in this age low sebum production so its decrease saturated fatty acids that provide a natural protective mechanism against dermatophytosis [36].

This result also showed that the causative agents were cultured in 301 cases of which 183were female patients and 118were male patients. Infections occurred more prevalent in females 60.7% (183/301) than males 39.3% (118/301) but not significantly different (P=0.105). This is similar with the study conducted in Libya by Eillabib *et al.* Infections occurred more frequently in females 65.1%than males34.9% [37], Dogo *et al.* who reported that the occurrence of infection among girls were higher (51.4%) than boys (41.5%) [22]. The reason for higher prevalence in female might be tight braiding of hair, hair dressing, the use of oil which may promote the disease [15].

Our study showed that dermatophyte pure isolated were *Trichophyton violaceum* 86% followed by *Trichophyton mentagrophytes* 5.88%,*Microsporum audouinii* and *Trichophyton tonsurans* shad the equal incidence rate 2.94%, *Trichophyton verrucosum* 2.2%respectively.Ourstudy finding indicated that *Trichophyton violaceum* the predominant dermatophyte as causative agents of Tinea Capitis. Our result relating to incidence of *Trichophyton violaceum* was corroborated by previous reports of Woldeamanuel *et al* .in Tulugudu. 80.6%[27], Ali *et al*. in Ethiopia (Gondar) 86.2% [38]. The frequency of etiological agents varies according to the region in rural southern Ethiopia. Perez-Tanoira *et al.* who report *Trichophyton verrucosum* was the most isolated[9].Also, in other countries Azab *et al*. who reported *Trichophyton violaceum* most isolated 40.3% in Egypt [39], Puri *et al.*74% in India [16]. Omar *et al*. found that *Trichophyton violeceum* was the only dermatophyte isolated (100%) in Alexandria [40]. *Trichophyton mentagrophytes* was isolated in 5.88% among dermatophyte which correlates with Moto *et al.* who reported 6.7% in Kenya [26].Anthropophiles commonly associated with poor hygiene, overcrowded living conditions and low socio-economic status [12].

Non-dermatophyte organisms are becoming increasing widespread. This noticeable coming out might be an artifact of improved diagnostic techniques and increased awareness that these fungi are potential etiologic agents. It is significant to accept that all isolated organisms should be recognized as potential pathogens when diagnosing fungal infections [40].

In our study, non-dermatophyte molds were isolated from 40.2% culture positive study subjects. This result closely related with Ndako*etal.*41.8% [24]. Among non-dermatophyte molds *Aspergillus fumigates* predominant filamentous fungi isolated. Our results agree with the finding of Adefemi *et al* [21] and Khaled *et al.* [20].

In Nigeria study done by Dogo *et al* found out that mycologically positive cases73.3% single infection and 26.7% mixed infection from sample of 100 children [22]. This result supports our findings of 85.4% single infection and 14.6% mixed infection observed. Another study by Grover *et al.* In India reported 10%of mixed infection cases from a total sample of 100 school children [24].

According to the literature most of them Tinea Capitis usually caused by dermatophytes [14–18] but in our study also non-dermatophyte cause Tinea Capitis. This may suggest that it is important to recognize non-dermatophyte for diagnosis.

8. Strengths and Limitations of the Study

8.1Strengths

The findings of the study isolate different species of dermatophuyte and non dermatophyte; the study addresses potential fungal pathogens.

8.2Limitations

Risk factors and drug susceptibility test were not performed in this study.

9. Conclusion and Recommendations

9.1 Conclusion

In conclusion, Tinea Capitis is a major health problem for a developing country like Ethiopia. From our study, we revealed high prevalence of Tinea Capitis infection with dermatophyte and non-dermatophyte. These fungal pathogens were associated with single and mixed infections. *Trichophyton violaceum* was the predominant dermatophyte fungi group isolated during our study. It was followed by non-dermatophytes the spectrums of fungi causing Tinea Capitis. There was a high significant correlation between1 to 14 age groups and prevalence of Tinea Capitis but there was no association between gender and fungal infection. Our study concluded that Tinea Capitis affects any age group but the highly affected age group was children between 1 to 14 years.

Our study indicates the importance of culture in diagnosis of all suspected Tinea Capitis cases. We believe that our results are useful in the developments of preventive and educational strategies.

9.2 Recommendations

- ✓ We recommend that direct microscopy and fungal culture can be done together. This will allow patients to receive proper diagnosis and early treatments

- ✓ Our study has shown that the prevalence of fungal infection is high in children. Therefore, we recommend minimizing the prevalence of Tinea Capitis by society health awareness regarding the cause of the disease, prevention and way of transmission also on time management of Tinea Capitis.

- ✓ We believe that our study provides baseline information. Therefore, we recommend further study for fungal agents and risk factors

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11. Annexes

Annex I: Questionnaires

Socio- Demographic characteristics of the Study participants.
1. Age _____

2. Sex 1) Male 2) Female

Comments_____

Name of principal investigator _____

Signature _____ Date _____

Annex II: Data record format

Socio- Demographic and data record format Addis Ababa University Collage of Health Sciences School of Nursing and Midwifery department of laboratory Science socio-demographic and record format or the P revalence of Tinea Capitis and spectrum dermatophytes and non-dermatophyte molds implicated in causing the mycosis among patients from at Rank Dermatology Clinic, Addis Ababa, Ethiopia

Sample code ------- Age----------

Gender

1	Female	2	Male

Site of infection (clinical manifestations) scalp

Microscopic (KOH) lab result:

1	Fungal element seen	2	No fungal element seen

Culture result of mycological agent_____

Annex III: English version of participant information sheet, consent/assent

Participant information sheet

Addis Ababa University, College of Health Sciences, department of laboratory, Addis Ababa University, Addis Ababa, Ethiopia

Title: Tinea Capitis: prevalence and spectrum dermatophytes and non-dermatophyte molds implicated in causing the mycosis among patients attending at Rank Dermatology Clinic, Addis Ababa, Ethiopia

Introduction

First of all we would like to thank you in advance for your cooperation and consent in participation in this study. Please listen when it is read for you about the general information of the study. If you have any question regarding the study please ask freely.

Back ground information: During the past 3 decades, the incidence of Tinea Capitis has increased worldwide with an estimated prevalence of 10% in the developed world and expectedly higher rate in most developing countries.

Aim of the study: The objective of this research is to determine the prevalence of Tinea Capitis and spectrum of dermatophytes and non-dermatophyte molds implicated in causing the mycosis among patients attending at Rank Specialized Dermatology Clinic, Addis Ababa, Ethiopia.

Procedure of the sample collection

The patients will be asked to come after head wash so as to remove any oil from the scalp. For all the patients, skin scrapings and hair fragments will be collected from the affected areas in an aseptic manner. The material collected will be prepared smears for microscopic and culture examination.

Time required for participating

You will spend 10-15 minutes until the specimen is collected, the questionnaire is filled and the consent is signed.

Benefits for participants:

Study participants will not have any financial incentives or other inducements from participating on this study. However, their results will be given and will be treated by the prescribing physician based on the KOH mount results and depending on the nature of the disease. Based on the physician's decisions, patients may be appointed to await culture results for better treatment.

Risks and complication

There is no considerable risk to the study subjects in participating in the study.

Confidentiality

In order to maintain the confidentiality of participants 'information, the name will not be given and the samples will be coded. Participants will not be prohibited to stop or withdraw at any time from the study. Only interested participants can retrieve their own lab result using their code number. The physician will be responsible for the interpretation of the results and providing treatment. No personal identifier will be disclosed to third party or will not appear in any report from this study.

Rights of the study participant.

Your participation is based on to make your decision by your own interest. If there is any unclear point, ask question until you understand and if want refuse or leave from the study at any time. Refusal to participate will not result in loss of medical care. or any other profit. You can obtain your results of the examination.

Communication

If you have any comment and suggestion about the project, contact addresses are:

Project Manager: Betelhem Yilma (BSc, MSc student), AAU

E: mail:beti.y2468@gmail.com

Cell phone +251-927347100

Advisor: Adane Bitew (PhD), DMLT, AAU

Cell phone: +251911039162

2. Consent form for (ages older than18 years old)

The objective and the application of the study were briefly clarified to me. I am also informed that my demographic and clinical data will be used for this research purpose from the laboratory request form and they will be kept confidential. Furthermore, I have been well informed of my right to refuse information, reject to collaborate and drop out of the study if I want and none of my actions will have any bearing at all on my overall health care. It is therefore with full understanding of the situation that I agreed to give the informed consent voluntarily to the researcher to give my specimen for the mentioned study.

Based on the above information I agree to participate in the research

Participant name----------Signature/fingerprint: ---------- Date----------
Witness's name----------signature: ----------Date ----------
Investigator's name---------- signature: ---------- Date ---------

3. Parental/Guardian Ascent Form for Ages Less than 18Years Old

I was in formed take whatever time I need to discuss the study with my family and friends, or anyone else I wish to. The decision to let my child join, or not join, is up to me, and will take him/her about10minutes, it is not painful and my child can stop participating at any time and will not lose any benefits as thereof.

As parent or legal guardian, I assure in my signature to become my child a participant in the research study described in this form.

Guardian's name----------Signature/fingerprint: ---------- Date----------
Witness's name----------signature: ----------Date ----------
Investigator's name---------- signature: ---------- date---------

Annex IV: Amharic Version (የአማርኛ ዉርስ ትርጉም)

ነ.የተሳታፊዎች መረጃ

የህክምና ላብራቶሪ ሳይንስ ክፍል ጤና ሳይንስ ኮሌጅ

የአዲስ አበባ ዩኒቨርስቲ አዲስ አበባ ኢትዮጲያ

የጥናቱ ርዕስ፡-

በራንክ የቆዳ ህክምና ክሊኒክ የሚመጡ ታማሚዎች ላይ በፈንገስ የሚታመሙ ሰዎች ብዛት መስፋፋት አዲሰ አበባ ኢትዮጲያ፡፡

በመጀመሪያ በጥናቱ ላይ ለመሳተፍ ፍቃደኛ ስለሆኑ ልባዊ ምስጋናዩ አቀርባለሁ፡፡ እባክዎን የተሳታፊዎች መረጃ ከልብ እዲያዳምጡ በትህትና እናጠይቃለን፡፡

ስለጥናቱ ያለዎትን ጥያቄ በማንኛዉም ጊዜ መጠየቅ ይችላሉ

ስለጥናቱ መረጃ፡-

በፈገስ የሚጠቀዉ የሰዉነት ክፍሎች መካከል ዋነኛዉ ፀጉር ነዉ

አብዛኛዎቹ ጥናቶች እደሚያሳዩት ነዐ ፐርሰንት የሚሆኑ ያለማችን ህዝቦች በዚህ በሽታ ይጠቃሉ ::በተለይ ሁኔታ በማደግ ላይ ባሉሀገሮች በመስፋፋት ላይ ይገኛል፡፡

የጥናቱ አላማ፡-

በራንክ የቆዳ ህክምና ክሊኒክ የሚመጡ ታካሚዎች ላይ በፈንገስ የሚጠቁ ሰዎች ብዛት መስፋፋት፡፡

የናሙና አሰባሰብ ሂደት

ናሙና የመስጠት ፍቃደንነት የሚጠይቅ ተመራማሪዉ ሲሆን የተገለፀዉን የሰዉነትክፍል ፖ አልኮል ከፀዳ በኃላ ናሙና ይወሰዳል ::ናሙናዉም ለሃኪም የሚረዳ ፤የሚያገለግልና የተሳታፊ ፍቃደኝነት ከተረጋገጠ በኃላ የተረፈዉን ናሙና ደግሞ ለጥናቱ የሚያገለግል ይሆናል፡፡

የጥናቱ ተሳታፊዎች ጥቅም፡-

ተሳታፊዎች በጥናቱ መሳተፍ ምንም አይነት የገንዘብ ጥቅም አያገኙም ነገር ግን ተሳታፊዎች የለሑሀ ምርመራ ና ዉጤት ተቀብለዉ ተገቢዉን ህክምና በሃኪማቸዉ በኩል እደበሽታዉ ሁኔታ ና እንደሃኪም ዉሳኔ ለተሸለ ህክምና ዉጤት ጠበወቀዉ እዲታከሙ ይደረጋል፡፡

ከጥናቱ ሊመጡ የሚችሉ የጎኒዮሽ ጉዳቶች፡-

በዚህ ጥናት የሚሳተፉ ሰዎች ምንም አይነት ጉዳት የማይደርስባቸዉ መሆኑን እንገልፃለን ::

የጥናቱ ሚስጢራዊነት፡-

የተሳታፊዎችን መረጃ ሚስጢራዊነት ለመጠበቅ ይረዳ ዘንድ የጥናቱ ተሳታፊዎች ስም በጥናቱ ላይ አይገለጽም:: በስም ፈንታ መረጃዎቹ በሚስጢራዊ ቁጥር /ኮድ/ ይመዘገባሉ::

እዲሁም ተሳታፊዎች በፈለጉት ሰዓት ከጥናቱ መዉጣት ይችላሉ:: ፍቃደኛ የሆኑ ታካሚዎች የሚሰጣቸዉ ኮድ ዉጤታቸዉን ማየት ይችላሉ::

ጥናቱን የሚያካሂደዉ ሰዉ ማረጋገጫ፡-

ለዚህ ጥናት ሃላፊነትን ለሚወስድ ማንኛዉም ጥናቱን የሚመለከት ጉዳይ ክትትል ለማድረግ እና ለሚመለከተዉ አካል መግላጫ ለመስጠት በፊርማያ አረጋግጣለሁ::

ቤተልሄም ይልማ

ፊርማ----------ቀን----------

ስልክ 0927347100

ነ.የፍቃደኝንት ማረጋገጫ ቅፅ/ከ 18 አመት እድሜ በላይ ለሆኑ/

የጥናቱ ርዕስ፡-

በራንክ የቆዳ ህክምና ክሊኒክ ለቆዳ ህክምና የሚመጡ ታማሚዎች ላይ በፈንገስ የሚታመሙ ሰዎች ብዛት አዲስ አበባ ኢትዮጲያ

ጥናቱ አላማ በራንክ የቆዳ ህክምና ክሊኒክ ለቆዳ ህክምና ለሚመጡ ታካሚዎች ላይ በፈንገስ የሚጠቁ ሰዎች ብዛት ላይ መሆኑ ተነግሮኛል ተገልጾልኛል:: ከዚህ ሌላ እኔ የምሰተዉ መረጃ ምስጢራዊ እንደሚሆን ተገልጸልኛል:: ከጥናቱ በፊለጉ ግዜ መዉጣት እንደምንችል ና ከጥናቱ በመዉጣቴ ምንም አይነት ጉዳት እንደማይደርስብኝ ተገልጾልኛል::

ይህን ከተረዳዉ በኃላ ለተመራማሪዉ ለመስጠት ፍቃደኝነቴን እገልፃለሁ::

የጥናቱ ተሳታፊ ስም ----------ፊርማ----------ቀን----------

የአጥኒዉ ስም----------ፊርማ----------ቀን----------

የምስክር ስም----------ፊርማ----------ቀን----------

የወላጅ ወይም ያሳዳጊ ፍቃደኝነት ቅፅ/ከ18 አመት እድሜ በታች ያሉ ታዳጊዎች ብቻ/

የጥናቱ ርዕስ፡-

በራንክ የቆዳ ህክምና ክሊኒክ ለቆዳ ህክምና የሚመጡ ታማሚዎች ላይ በፈንገስ የሚታመሙ ሰዎች ብዛት አዲስ አበባ ኢትዮጲያ

በዚህ ጥናት ዉስጥ የእርሶ ልጅ ስለተመረጠ እባኮዎን ስለልጅዎ በዚህ ጥናት የመሳተፍ ፍቃደኝነትዎን ያሳዉቁን ዘንድ እርስዎ ፍቃደኛ ከሆኑ ልጅዎ ከተገለፀዉ የሰዉነት ክፍል ናሙና እንድንወስድ የጎዉም ከ10 ደቂቃ በላይ የማይወስድ መሆኑን ና ህመም የሌለዉና እንዲሁም በፈለገዉ ግዜ ከጥናቱ መዉጣት እንደሚችል በመዉጣቱም ምንም ጉዳትና ከህክምናም ምንም ጉዳት እንደሌለ እንገልፃለን፡፡

የአሳዳጊዉ /የወላጅ ስም----------ፊርማ----------ቀን----------

የጥናቱ ተሳታፊ ስም----------ፊርማ ----------ቀን----------

የአጥኒዉ ስም ----------ፊርማ----------ቀን----------

የምስክር ስም ----------ፊርማ----------ቀን----------

Annex V: Reagent preparation (Stains and Media)

A. 10% POTASSIUM HYDROXIDE

Formula; Potassium hydroxide (KOH) 10g

Glycerol ……20ml

Distilled Water…. 80 ml

Weight 10g KOH pellets, transfer the chemical to a screw cap bottle. Then add 80 ml distilled water and mix it until completely dissolved. Add 20 ml glycerol and label the bottle and make it corrosive.

Purpose: To digest organic material e.g., tissue cells in a specimen in order to visualization of fungi to be more easily demonstrated.

Principle: The KOH mount is used to aid in detecting fungal elements in specimens containing keratinous material. The KOH dissolves the background keratin, unmasking the fungal element to make them more apparent. Dissolving is improved by gently heating. Glycerol used for prolongs shelf-life by preventing crystallization.

Procedure

1. Place a drop of 10% KOH solution on a slide.
2. Transfer small amount of the specimen to drop of KOH, apply cover slip and heat on slide warmer and allow to stand 5 up to 10 min place the petri dish with a cover, together with a filter paper to prevent the preparation from drying out.
3. Observe under low light microscope.

B. Lacto phenol cotton blue (LPCB)

Formula a preparation of 50mlLPCB

- ✓ Distilled water…….50ml
- ✓ Lactic acid……. 50 ml.
- ✓ phenol crystals…...50 g.
- ✓ Cotton blue…….0.125g.
- ✓ Glycerol……... 100 ml.

Purpose: To identification of fungal cell walls

Principle: fungi are eukaryotic organisms with both macroscopic and microscopic characters. The fungal spore cell wall which the component of LPCB stain for identification. This staining agent made up of three components. Phenol acts as disinfestation by killing any living organism,

lactic acid to preserve the fungal structures, cotton blue to stains fungal cell walls. Stain present blue colored.

Preparation of Lacto phenol cotton blue

1. Put a drop of 70 % alcohol on microscope slide.
2. Small piece from cultures of filamentous fungi should be removed using inoculating wire.
3. Flam the wire until the whole length of wire glows red hot and ensure wire has cooled.
4. Open the petri dish and remove a small amount of the culture, transferred to the slide.
5. Tease out the material very gently with mounted needles.
6. Lower the cover slip gently on to the slide.
7. Examination using a low power objective lens.

C. Sabouraud Dextrose Agar with Chloramphenicol and Gentamicin

Formula

Distilled Water……..1000ml

Dextrose…….....40 g

Peptone……..…….40 g

Agar.....15.0g

Chloramphenicol.....0.05 g

Gentamicin …….40 mg/ml

Storage Instructions: store plates in the dark at 2 – 8°C ready for use.

Annex VI: Principles of the procedure

Sabouraud Dextrose Agar is used for the isolation and cultivation of fungi. Supplemented with dextrose to support the growth of fungi. The peptones provide the sources of nitrogenous compound and nutritious source of amino acidrequired for fungi, yeasts and molds growth in SDA. Dextrose is the fermentable carbohydrate in corporate in high concentration as a carbon and energy source provides for the growth of microorganisms. Agar is the solidifyingagent. In addition of Chloramphenicol act as broad-spectrum antibiotic to inhibit the growth of wide range of gram-negative and gram-positive bacteria. Gentamicinwas added to further inhibit the growth of gram-negative bacteria.

Procedure

1. Combine all ingredients in 900ml of d water
2. Adjust to PH 5.6 with hydrochloric acid and adjust final to 1 liter
3. Heat to boiling point and stirring well to dissolve the medium completely.
4. Add the Gentamicin and autoclave at 121°C for 15 minutes.
5. Cool to 45 to 50°C and pour in to sterile petri dishes
6. For the processing of specimens, streak the specimen on to the medium.
7. Plates were incubated the plates at 25-30°C in an inverted position with increased humidity

D. Potato Dextrose Agar with chloramphicol

Composition

Potato Extract…….200.0Gms/L

Dextrose…….20.0Gms/L

Agar……15.0Gms/L

Purpose: used for growing clinically significant yeast and mold.

Principle

Potato dextrose agar contains dextrose as a carbohydrate source which serves as growth stimulant and potato infusion that provides a nutrient base for luxuriant growth of most fungi. Agar added as solidifying agent.

Preparation of potato Dextrose Agar

1. To prepare potato infusion, boil 200g sliced, unpeeled potatoes in l liter distilled water for 30 min.
2. Filter through cheesecloth, saving effluent
3. Mix with Dextrose, Agar and Water then boil to dissolve and autoclave 15 min at121°C
4. Dispense 20 to25 ml portions in to sterile petri dishes

E. Mycosel Agar

Dehydrated Mycosel Agar……36g.

Distilled Water……1000ml.

Mix thoroughly. Heat with frequent agitation until medium boils and autoclave 15 min at121°C

Purpose To isolate pathogenic fungi

Principle

The nutritive properties ofMycosel Agar are supplied by the peptone prepared from soybean meal. Dextrose is an energy source for metabolism of fungi. cycloheximide inhibits most saprophytic molds.

F. UREASE TEST

Urease test medium

Urea agar base …….29g

Agar ……15g

Distilled water …….1000ml

Principle

Urea medium, agar contains urea and the phenol red as PH indicator. Many organisms which catalyze the splitting of urea in the presence of water to release two molecules of ammonia and carbon dioxide. The NH3combines with carbon dioxide and water to form ammonium carbonate, which convert the medium alkaline, orange yellow color to converting in color of indicator to reddish pink. The change of the color due to the PH indicator if the color change to reddish pink it is positive eg. *T. mentagrophytes*, unchanged colors of media it is negative.

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of supplies used for the thesis have been duly acknowledged.

M.Sc. candidate: Betelhem Yilma (B.Sc.).

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisor.

Advisor:

Adane Bitew (MSc, PhD).

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.